REMARKS

Applicants thank Examiner Venci and Examiner Le for the courtesy of conducting the telephonic interview with the undersigned on August 9, 2005, and particularly for their helpful suggestions regarding claim amendment.

I. The Invention

The present invention relates to a new method for measuring the amount of cholesterol present in different species of lipoproteins, such as low density lipoprotein (LDL) and high density lipoprotein (HDL), as well as the total amount of cholesterol in a sample. In this method, a complex comprising a first lipoprotein fraction (*e.g.*, LDL) is formed and therefore renders the cholesterol associated with this lipoprotein fraction unavailable for measuring. The amount of cholesterol not involved in the complex (*i.e.*, cholesterol associated with non-LDL) is then measured. Subsequently, the complex is dissolved, making the cholesterol associated with the first lipoprotein fraction available for measuring, and the amount of total cholesterol in the sample is then measured. Through subtraction, the amount of cholesterol associated with the first lipoprotein fraction can be determined. The claimed method provides a novel means for measuring the amount of cholesterol associated with different lipoproteins in the same test tube.

II. Status of the Claims

Claims 1-29 were originally filed and claims 22-29 were later canceled. Upon entry of the present amendment, claim 1 specifically recites that the first and second lipoprotein fractions are two different fractions and that the complex-forming agent used in step (a) forms a complex with the first lipoprotein fraction but not with the second fraction. Claim 1 is further amended to clarify how the amount of cholesterol associated with the first lipoprotein fraction is determined. This amendment is supported by the specification, for example, on page 7, line 21, to page 9, line 17, where these features of the claimed method are described. In addition, claims 17, 18, and 21 are amended to correct typographic errors where proper spacing between certain words was missing.

The present amendment was not presented in Applicants' last response, because Applicants in good faith believed that the last response, without this amendment, would be sufficient to overcome the outstanding rejections. Since this amendment adds no new matter and requires no new search, its entry is respectfully requested.

III. Claim Rejections

A. 35 U.S.C. §112, Second Paragraph

Claims 1-6 and 8-21 were again rejected under 35 U.S.C. §112, second paragraph, for alleged indefiniteness. Applicants respectfully traverse the rejection in light of the present amendment.

"Fraction"

In the final Office Action mailed May 17, 2005, the Examiner contended that the word "fraction" renders claims 1, 4-6, 17, and 19 indefinite, because the claims do not allude to "the existence of more than one species of lipoprotein" or "the notion that determination of 'fraction' [is] based on a different interaction with the recited 'complex-forming agent" (bottom of page 6 of the final Office Action). As amended, claim 1 specifically recites that, first, there are two different lipoprotein fractions--a first lipoprotein fraction and a second lipoprotein fraction in the sample; and second, the complex-forming agent added into the sample forms a complex with the first lipoprotein fraction, but not with the second lipoprotein fraction. Applicants believe that this amendment adequately addresses this particular concern.

"A First Cholesterol Value"

The Examiner also asserted that the phrase "a first cholesterol value" in claim 1, step (b), is indefinite because its purpose 'remains confounding" (middle paragraph on page 7 of the final Office Action). Claim 1 is now amended to delete this phrase. Furthermore, Applicants wish to clarify the following: first, the phrase "first cholesterol value," although no longer used, corresponds to the amount of cholesterol associated with a second lipoprotein fraction, which is determined in step (b). Second, the amount of total cholesterol in a sample is determined in step

(d), after the complex of the first lipoprotein fraction and the complex-forming agent is disrupted. Thus, although the amount of cholesterol associated with the second lipoprotein fraction (as determined in step (b)) does not directly participate in the calculation of the total amount of cholesterol, the amount of cholesterol associated with the second lipoprotein fraction is taken into account of the total amount of cholesterol. This is due to the accumulative nature of the cholesterol-measuring reactions, which can be a colorimetric or absorbance-based method. Third, the amount of cholesterol associated with the second lipoprotein fraction is directly involved in the calculation of the amount of cholesterol associated with the first lipoprotein fraction: the latter is obtained by subtracting the former from the amount of total cholesterol in a sample. These features would be clear to a person of skill in the art upon reading the present disclosure.

"Measuring the Amount of Cholesterol in Steps (b) and (d)"

Claims 10 and 11 were further rejected for alleged indefiniteness for reciting the enzymatic methods useful for determining either the amount of cholesterol associated with a lipoprotein fraction, or the total amount of cholesterol. Specifically, the Examiner asserted that accumulative nature of a colorimetric or absorbance-based method is not apparent based on the claim language.

In response, Applicants wish to point out the following: first, the accumulative nature of the cholesterol measuring method was brought up as a specific answer to the Examiner's specific question as to how the total cholesterol amount could be measured in step (d) when apparently a portion of the cholesterol (the portion not sequestered in the complex with the complex-forming agent) had already been used up in step (b). Second, whatever specific method is used for measuring cholesterol quantity, the language of step (d) describes the only logical manner to calculate the amount of cholesterol associated with the first lipoprotein fraction: by subtracting the amount of cholesterol associated with the second lipoprotein fraction from the total amount of cholesterol. In other words, the calculation defined by the language of step (d) is correct, independent of the specific manner cholesterol level is measured. Since a person of

ordinary skill in the art would be familiar with various suitable methods for quantifying cholesterol, he/she would readily recognize the above discussed points, especially after reviewing the instant disclosure. There is not ambiguity associated with this particular language of claims 1, 10, and 11.

For these reasons, the withdrawal of the indefiniteness rejections under 35 U.S.C. §112, second paragraph, is respectfully requested.

B. 35 U.S.C. §102

Claims 1-6 and 8-21 were rejected under 35 U.S.C. §102(e) for alleged anticipation by Miki *et al.* (U.S. Patent No. 6,162,607). Applicants respectfully traverse the rejection.

To anticipate a pending claim, a prior art reference must provide, either expressly or implicitly, each and every limitation of the pending claim. MPEP §2131. The pending claims are directed to a method for sequentially determining amounts of cholesterol in two different lipoprotein fractions present in a sample, as well as the total amount of cholesterol in the sample. This method involves sequestration of the first lipoprotein fraction, determination of cholesterol quantity in the second, unsequestered lipoprotein fraction, release of the sequestered first lipoprotein fraction, and determination of total amount of cholesterol quantity in the sample. These measurement steps plus calculation provide the amounts of cholesterol in the two different lipoprotein fractions as well as the total amount of cholesterol.

In contrast, the Miki *et al.* references relates to a method for measuring the amount of a constituent (*e.g.*, cholesterol) in only one lipoprotein fraction, but not the amount of the constituent in the remaining fraction or the total amount of the constituent, in a sample. The Miki *et al.* method includes the following steps:

First, a mixture is made by adding into a sample an antibody, which binds specifically to the lipoproteins other that the specific species of lipoprotein to be measured.

Second, the optical absorbance (OD_1) of the mixture from the first step is obtained.

Third, a reagent capable of causing a reaction for measuring the constituent (*e.g.*, cholesterol) is added to the mixture from the first step, and the optical absorbance (OD_2) is obtained upon completion of the reaction. An exemplary reaction for this step is the reaction between cholesterol and cholesterol oxidase.

Fourth, the amount of constituent (*e.g.*, cholesterol) is determined based on the difference between OD_1 and OD_2.

See, e.g., column 2, lines 10-25; and column 6, lines 15-26, of the Miki *et al.* reference. Thus, when OD_1 is measured following the first and second steps, this absorbance value reflects merely the background reading of the antibody-lipoprotein complex, because no cholesterol-measuring reaction has taken place at that time. When OD_2 is obtained in the third step following the cholesterol-measuring reaction, the ***increase*** in absorbance reading reflects the amount of cholesterol present in the species of lipoprotein(s) not a part of the antibody-lipoprotein complex. The subtraction of OD_1 from OD_2 therefore only serves the purpose of eliminating background signal in the process of determining the amount of cholesterol of one fraction of lipoprotein(s) in a sample. The Miki *et al.* reference does not teach how to determine the amount of cholesterol present in the remaining lipoprotein fraction or the total amount of cholesterol in the sample.

In the final Office Action, the Examiner asserted that the Miki *et al.* reference anticipates the claimed invention of this application, because the reference teaches the inclusion of a surfactant at a level of up to 10 w/v % in a sample before measuring the amount of cholesterol, which would lead to the release of all lipoprotein species from a complex formed with an antibody (second paragraph on page 8 of the final Office Action) and therefore allow the measure of total cholesterol amount in a sample. Applicants cannot agree with the Examiner for the reasons specifically discussed below.

First of all, the purpose of Miki *et al.* is to measure the amount of cholesterol associated with a particular lipoprotein, not to measure the total cholesterol in a sample. This is

evidenced by the description throughout the specification, for example, in the Abstract, where it is stated, "[t]here is provided a method and kit for measuring the amount of an objective constituent ***contained in a specific lipoprotein*** in a biological sample such as serum and plasma, specifically for measuring the amount of cholesterol contained in high density lipoprotein, which can be applicable to clinical tests." Also in column 2, lines 39-45, it is stated that "[t]he present inventors have made an extensive research work for finding a method for measuring directly the amount of an objective constituent ***contained in a specific lipoprotein*** by use of an automatic analyzer ***without carrying out any pretreatment operation to remove the lipoproteins other than the specific lipoprotein***." In column 2, lines 46-52, it is further stated that "the inventors have found that measurement of constituent(s) ***in a specific lipoprotein without previously removing lipoproteins other than the specific lipoprotein*** is possible by mixing a biological sample with ***a first reagent solution comprising an antibody reactive to lipoprotein(s) other than the specific lipoprotein*** to allow a reaction to take place..."

The highlighted language, consistent throughout the entire specification of Miki *et al.*, provides unequivocal indication that the teaching of the reference relates to measuring the cholesterol associated with one specified type of lipoprotein only.

The Miki method is not intended to measure total amount of cholesterol in a sample also for the simple reason of lack of necessity--if the quantity of total cholesterol in a sample were to be measured, then there would be no reason at all to follow the teaching of Miki *et al.* and go through the painstaking steps of adding antibody to sequester lipoprotein species other than the specific one(s) whose cholesterol content is to be measured; the measurement of total cholesterol could be easily accomplished by directly using methods well known to those of skill in the art

Second, a complete reading of the sections in the Miki reference the Examiner pointed to as evidence of disruption of antibody-lipoprotein complex by a surfactant reveals that the surfactant is intended for a completely different purpose. In column 5, lines 58-62, where the Examiner has cited the up to 10 w/v % surfactant level, it is stated that the surfactant is included

in "the 2nd solution." The purpose of this "2nd solution," according to column 5, lines 30-34, is to "promot[e] the measurement reaction of the objective constituent contained in the specific lipoprotein, particularly cholesterol, so as to make it possible to shorten the reaction time." To add a surfactant so that it disrupts the antibody-lipoprotein complex resulted from the mixing of a sample with a first reagent solution comprising antibody simply defeats the fundamental purpose of the Miki method as described in column 2, lines 46-60.

Third, even if the Examiner were correct in that the presence of a surfactant reaching the concentration of 10 w/v % could partially disrupt the antibody-lipoprotein complex, as the description in column 5, lines 64-67, appears to suggest, such partial disruption by definition would not allow the measurement of total cholesterol amount in a sample.

For these three reasons, the addition of a surfactant is not intended to and certainly should not significantly disrupt the complex between antibody and lipoprotein for the basic purpose of the Miki method. Accordingly, no total cholesterol is measured by the Miki method.

There is a fourth reason why the Examiner's position is untenable. Even if the presence of a surfactant at the level of 10 w/v % were to completely disrupt all complex formed between the antibody and lipoproteins and thus allowed the measurement of total cholesterol amount in a sample, the Miki method would then fail to provide a different limitation of the pending claims: it would not be measuring the amounts of cholesterol associated with two different lipoprotein fractions. From a logical perspective, the antibody-lipoprotein complex in the Miki reference is either completely disrupted, in which case the Miki method cannot measure the cholesterol amounts associated with two different lipoprotein fractions; or the antibody-lipoprotein complex is not disrupted / incompletely disrupted, in which case the Miki method cannot measure the total cholesterol amount. In short, the Miki method cannot measure **both** the total amount of cholesterol and the amounts of cholesterol in two different lipoprotein fractions sequentially, as required by the pending claims. The Miki reference therefore cannot anticipate

the claims of the instant application. Applicants thus respectfully request the withdrawal of the anticipation rejection.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

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